

AMENDMENTS

IN THE SPECIFICATION

On page 1, under the heading "PHOTON REDUCING AGENTS FOR FLUORESCENT ASSAYS", please delete the sentence,

"This application claims the benefit of priority under 35 U.S.C. §119(e) to United States provisional application No. 60/054,519, filed 8/1/97."

In its place please insert the text below.

--This is a continuation of United States patent serial number 09/122,477 filed July 23, 1998, now allowed, ^{as US Patent Number 6,231,612} which claims the benefit of U.S. Provisional Application No. 60/054,519 filed August 1st 1997.--

IN THE CLAIMS

Please cancel claims 1 to 73 and 78 to 79 and add claims 80 to 116 as below.

--80. A system for fluorescence assays, comprising:

- a) a source of excitation light, for fluorescent excitation;
- b) a detector, for measuring emission; and
- c) an aqueous sample, comprising:
 - i) a plurality of living cells in contact with a solid surface,
 - ii) a first reagent, comprising a photon producing agent,
 - iii) a second reagent comprising a photon reducing agent, wherein said photon reducing agent is substantially impermeant to a plasma membrane of a living mammalian cell,

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wherein said photon reducing agent does not specifically bind to said membrane compartment,

wherein said photon reducing agent has an absorption spectrum that overlaps with the absorption, emission or excitation spectrum of said photon producing agent, and

wherein said photon reducing agent is present in said aqueous sample at an amount sufficient to reduce light emitted from said aqueous sample by at least 10 % compared to the light emitted from said aqueous sample in the absence of said photon reducing agent.

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81.

The system of claim 80, wherein said solid surface is a bottom of a multiwell plate.

3.
82.

The system of claim 81, wherein said multiwell plate has between 6 and 3,456 wells.

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83.

The system of claim 81, wherein said multiwell plate has greater than 384 wells.

5.
84.

The system of claim 80, further comprising a temperature controller.

6.
85.

The system of claim 80, further comprising a multi-axis translation stage to move a multiwell plate.

7.
86.

The system of claim 80, further comprising auto focusing optics.

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Sub C1
~~8.~~
87. The system of claim ~~80~~, further comprising programmable control of imaging and data collection.

~~9.~~
88. The system of claim ~~80~~, wherein said plurality of living cells comprises a target receptor.

~~10.~~
89. The system of claim ~~80~~, wherein said plurality of living cells comprises a target ion channel.

Cut
~~11.~~
90. The system of claim ~~80~~, wherein said plurality of living cells comprises a target intracellular nuclear receptor.

Sub B2
91. The system of claim ~~80~~, wherein said photon reducing agent is present in said aqueous sample at an amount sufficient to reduce light emitted from said aqueous sample by at least 30 % compared to the light emitted from said aqueous sample in the absence of said photon reducing agent.

92. The system of claim ~~80~~, wherein said photon reducing agent is present in said aqueous solution at an amount sufficient to reduce light emitted from said aqueous sample by at least 50 % compared to the light emitted from said aqueous sample in the absence of said photon reducing agent.

93. The system of claim ~~80~~, wherein said photon reducing agent is present in said aqueous sample at an amount sufficient to reduce light emitted from said aqueous sample by between 70 and 99 % compared to the light emitted from said aqueous sample in the absence of said photon reducing agent.

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Sub C1
B2
~~15.~~
~~94.~~ The system of claim ~~80~~¹, wherein said photon producing agent is selected from the group consisting of a fluorescent enzymatic substrate, a fluorogenic enzymatic substrate, a member of a FRET pair, a molecule that detects voltage across a membrane of a membrane compartment and an intracellular ion indicator.

Cont
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~~16.~~
~~95.~~ The system of claim ~~94~~¹⁵, wherein said photon producing agent is a fluorescent enzymatic substrate.

~~17.~~
~~96.~~ The system of claim ~~94~~¹⁵, wherein said photon producing agent is a fluorogenic enzymatic substrate.

~~18.~~
~~97.~~ The system of claim ~~94~~¹⁵, wherein said photon producing agent is a member of a FRET pair.

~~19.~~
~~98.~~ The system of claim ~~94~~¹⁵, wherein said photon producing agent is a molecule that detects voltage across a membrane.

~~20.~~
~~99.~~ The system of claim ~~94~~¹⁵, wherein said photon producing agent is an intracellular ion indicator.

~~21.~~
~~100.~~ The system of claim ~~80~~¹, wherein said photon reducing agent is selected from the group consisting of a collisional quencher, a particulate, an absorption quencher, a FRET quencher and a dark complex.

~~22.~~
~~101.~~ The system of claim ~~100~~²¹, wherein said photon reducing agent comprises a particulate or colloidal quencher.

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102. The system of claim 80, wherein said photon reducing agent comprises a light absorbing dye.

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103. The system of claim 102, wherein said photon reducing dye is not a pH indicator dye.

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104. The system of claim 100, wherein said photon reducing agent comprises a FRET quencher.

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105. The system of claim 80, wherein said second reagent comprises at least two photon reducing agents.

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106. The system of claim 105, wherein said second reagent comprises Tartrazine.

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107. The system of claim 105, wherein said second reagent comprises chromotrope 2R.

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108. The system of claim 105, wherein said second reagent comprises Acid Fuchsin.

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109. The system of claim 105, wherein said second reagent comprises Patent Blue.

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110. The system of claim 105, wherein said second reagent comprises Acid Red 37.

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111. The system of claim 105, wherein said second reagent comprises chromotrope F8.

112. The system of claim 105, wherein said second reagent comprises Tartrazine.

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Sub C1
33.
113. The system of claim 80, wherein said photon reducing agent improves the optical signal to noise ratio by at least 300 % compared to the optical signal to noise ratio in the absence of said at least one photon reducing agent.

Cont 34.
a2 114. The system of claim 80, wherein the steady state concentration of said photon reducing agent within said plurality of living cells is less than 50 % of the concentration of said photon reducing agent outside of said plurality of living cells.

35.
115. The system of claim 80, wherein the steady state concentration of said photon reducing agent within said plurality of living cells is less than 30 % of the concentration of said photon reducing agent outside of said plurality of living cells.

36.
116. The system of claim 80, wherein the steady state concentration of said photon reducing agent within said plurality of living cells is less than 10 % of the concentration of said photon reducing agent outside of said plurality of living cells.--

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